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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

RESEARCH LETTER

Estrogen Receptor α Loss-of-Function Protects Female Mice From DSS-Induced Experimental Colitis

Males are at greater risk than females for developing ulcerative colitis (UC) and experiencing worse clinical disease[1–3]; the molecular basis for this sex bias remains unclear. An important regulatory mechanism of colonic homeostasis is via noncanonical estrogen receptor (ER) signaling. Very low levels of circulating estrogen are required to bind transmembrane and cytosolic ERs, such that immune responses in both sexes are subject to regulation by estrogen. Estrogen receptor β (ERβ) is expressed abundantly in the human colon,[4,5] where it has a critical role in maintaining barrier function and colonic architecture.[6,7] We therefore examined the in vivo functional effects of ERβ gain-of-function and loss-of-function using a dextran sulfate sodium-induced murine model of acute experimental colitis (DSS-AEC).

We challenged ERβ-deficient mice (ERβ-knockout [KO]), ERα-deficient mice (ERα-KO), or wild-type littermate controls (WT) with DSS-AEC and measured clinical parameters including weight loss (Figure 1*A*), disease activity index (Figure 1*B* and Supplementary Figure 1), colon length (Figure 1*C*), and total inflammatory scores including percentage ulceration, re-epithelialization, active and chronic inflammation, and transmural inflammation (Figure 1*D* and Supplementary Figure 2). We also performed experimental endoscopies[8] to assess inflammation and tissue damage (Figure 1*E*) and histologic assessment of DSS-treated colon tissues (Figure 1*F*). Interestingly, ERα-KO-male (M) mice lost the most weight of any group, whereas ERα-KO-female (F) mice lost very little weight (Figure 1*A*). ERα-KO-M also showed the most severe disease activity index scores (Figure 1*B*) and the most significant colon shortening (Figure 1*C*), with significant interaction effects between genotype and sex. Based on H&E staining of colon tissues, total inflammatory scores showed similarly exacerbated colitis among ERα-KO-M mice (Figure 1*D*). Experimental endoscopies showed that ERα-KO-F mice appeared nearly normal, whereas ERα-KO-M mice showed focal ulcerative lesions with spontaneous bleeding and loss of colon transparency (Figure 1*E*). Histologic assessment showed profound inflammation, epithelial erosion, and loss of tissue architecture in ERα-KO-M mice as well as ERβ-KO-F mice (Figure 1*F*).

ERβ has been shown to be a dominant-negative regulator of ERα-mediated signaling,[9] leading us to postulate that sex-specific differences in colonic gene expression of ERα or ERβ may underlie sex-based differences in response to DSS-AEC. Interestingly, we found that knockdown of each individual ER isoform results in compensatory up-regulation of the other (Supplementary Figure 3), a pattern that occurs to a similar extent in both sexes and is therefore unlikely to contribute to sex-based differences.

We next analyzed the potential differences in colonic gene expression between DSS-treated ERα-KO-M and ERα-KO-F mice using a polymerase chain reaction array of 84 known ER-regulated genes. All gene expression values were normalized to the *B2m* gene, and z-scores were calculated for all genes (full data set) (Supplementary Figure 4*A*). Trimming the data for genes that are significantly and uniquely different between ERα-KO-M and ERα-KO-F DSS-treated colon tissues (Supplementary Materials and Methods section and Supplementary Figure 4*B*) resulted in the identification of cathepsin D (*Ctsd*), *Fos*, and *Socs3*.

Gene expression of *Socs3*, *Ctsd*, and *Fos* was confirmed by traditional quantitative polymerase chain reaction in a larger colon tissue sample set from DSS-treated ERα-KO-M and ERα-KO-F mice. In agreement with the array data, all 3 genes showed higher expression among DSS-treated ERα-KO-M compared with ERα-KO-F mice (Figure 2*A*). Interestingly, *Ctsd* and *Fos* both showed sex-specific differences in gene expression after DSS-AEC: *Ctsd* expression was reduced significantly in ERα-KO-F mice, but unchanged in ERα-KO-M mice, whereas *Fos* expression was increased significantly in ERα-KO-M mice, but unchanged in ERα-KO-F mice (Figure 2*A*). Gene expression of *SOCS3*, *CTSD*, and *FOS* in UC patients or control colon biopsy specimens showed that *CTSD* expression was reduced in female UC patients compared with controls, whereas male UC patients and controls expressed similar *CTSD* levels (Figure 2*B*). In contrast, male UC patients expressed higher *FOS* compared with controls, whereas female UC patients and controls expressed similar *FOS* levels (Figure 2*B*). No significant difference between male and female control or UC patients in gene expression of ERα or ERβ (Figure 2*C* and *D*) was observed, suggesting that the differences observed in *Fos* and *Ctsd* are not owing to differential ERα/ERβ expression.

Our findings suggest that fundamental differences in ERα/ERβ signaling ratios impact colitis in males and females. Specifically, ERβ expression in female mice protected against DSS colitis, whereas it failed to protect male mice. Our findings provide insight toward potential mechanisms by which sex-based differences in intestinal inflammation arise. We propose that signaling downstream of ERα/ERβ results in differential gene expression in males vs females, ultimately leading to enhanced colitis in males. Improved understanding of the mechanisms by which loss of ERα signaling fails to protect males from colonic inflammation may eventually lead to more specific and efficacious UC therapies.

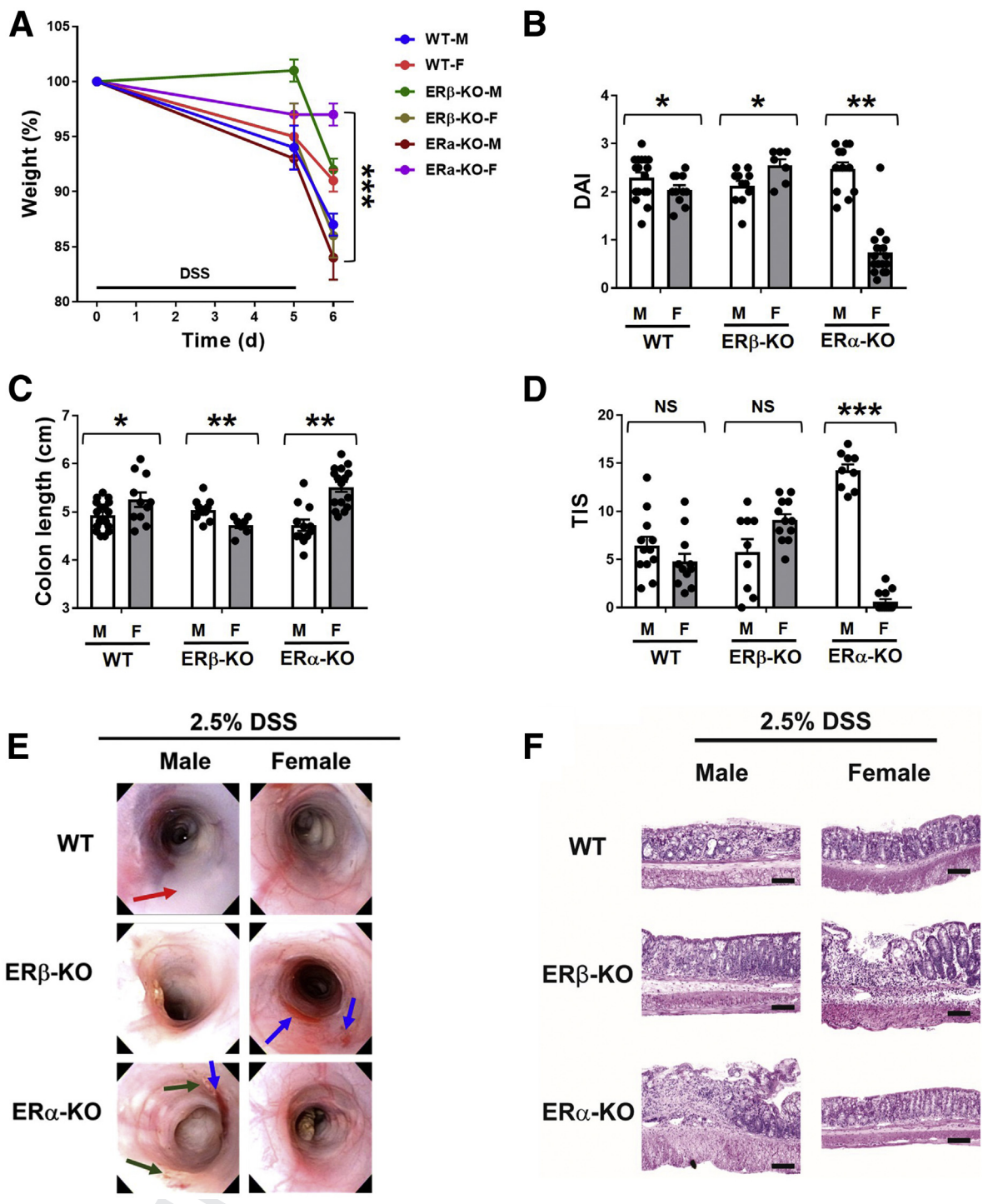

Figure 1. DSS-induced colitis in WT, ERβ-KO, and ERα-KO male and female mice. Ten- to 12-week-old male (M) and female (F) WT, ERβ-KO, and ERα-KO mice were fed 2.5% DSS-supplemented drinking water for 5 days and killed on day 6. (*A*) Body weights were recorded at days 0, 5, and 6 and are expressed as the percentage of initial (day 0) weight. (*B*) The disease activity index (DAI) was calculated for each mouse at day 6 (encompassing body weight loss, stool consistency, and hemoccult scores). Analysis of variance (ANOVA) $F = 36.3$; $P < .0001$; $\alpha = .05$ with 2 df. (*C*) Colon length was measured on day 6 (ANOVA $F = 12.4$; $P < .0001$, $\alpha = .05$ with 2 df). (*D*) H&E-stained colon tissues collected from mice on day 6 were assessed for total inflammatory scores (TIS, encompassing ulceration; re-epithelization; active and chronic inflammation; and transmural inflammation; ANOVA $F = 57.06$ for interaction effects; $P < .0001$; $\alpha = .05$ with 2 df). Data are represented as the means ± SEM of 7–17 individual mice/group; *dots* represent individual values. $^{*}P \leq .05$, $^{**}P \leq .01$, and $^{***}P \leq .001$. (*E*) Endoscopic evaluations were performed of the descending colon on day 6, immediately before death. *Arrows* represent loss of transparency (red), bleeding (blue), and focal edematous lesions (green). (*F*) Distal colon tissues were harvested for H&E staining. *Scale bar*: 10 μm; magnification, 10× + 1.25 NA.

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